Supplementary Materials

Table S1. List of characteristic spectral peak centers and amplitudes for (zero-order derivatives of) 3 seagrasses found within the coastal waters of metro-Adelaide, South Australia.

5 nm-increments	# seagrass found at this interval	0^{th} deriv. for *Posidonia spp.*		0^{th} deriv. for *Amphibolis spp.*		0^{th} deriv. *for Heterozostera spp.*	
		Center	Amplitude	Center	Amplitude	Center	Amplitude
415	2	414.06	0.008000			416.00	0.004611
450	1	451.18	0.013316				
485	1	483.39	0.038433				
490	1					489.96	0.013383
510	1			509.57	0.072113		
525	1	524.77	0.099000				
530	2			532.90	0.103127	530.93	0.073351
545	1	547.21	0.165883				
555	1			553.88	0.101248		
575	1					574.43	0.068167
585	3	583.03	0.106206	582.64	0.085488	583.95	0.060880
600	1			599.37	0.080094		
605	1	606.41	0.057722				
620	2	620.82	0.058420			620.00	0.033922
630	1			630.30	0.087652		
635	2	635.13	0.063558			636.58	0.029689
650	1	652.78	0.012750				
665	1					663.46	0.003387
710	3	707.49	0.051680	710.85	0.102476	707.62	0.014989
715	2	716.41	0.074350			716.52	0.016708
735	1	733.84	0.047002				
740	1			739.93	0.064128		
Total # Peaks		13		8		10	

Table S2. List of characteristic spectral peak centers and amplitudes for (first-order derivatives of) 3 seagrasses found within the coastal waters of metro-Adelaide, South Australia.

5 nm-increments	# overlapping	1st deriv. for *Posidonia spp.*		1st deriv. for *Amphibolis spp.*		1st deriv. *for Heterozostera spp.*	
		Center	Amplitude	Center	Amplitude	Center	Amplitude
415	2	415.25	6.3756E-04	417.13	1.0251E-03		
435	1			436.46	9.2946E-04		
440	1	438.90	3.4658E-03				
445	1					444.22	6.8778E-04
450	1			450.11	1.3914E-03		
455	2	455.63	3.2721E-03	456.26	3.5702E-03		
475	2	474.10	5.7009E-03	474.48	3.4200E-03		
480	1					477.70	1.9268E-03
490	2	491.97	6.8768E-03	490.87	4.8627E-03		
505	3	506.49	5.4763E-03	507.49	6.0244E-03	506.33	1.5723E-03
520	2	521.60	1.1575E-02	522.20	7.2212E-03		
525	1					524.68	3.2442E-03
530	1			531.27	2.0164E-03		
540	1	541.47	7.8191E-03				
545	1			545.14	5.0392E-03		
550	1					549.40	1.1757E-03
555	1	555.90	5.4282E-03				
560	1			558.35	3.6579E-03		
575	3	573.51	7.2536E-03	574.73	7.1175E-03	577.28	8.4304E-04
590	2	589.94	5.9642E-03	590.17	6.0018E-03		
600	1					598.78	7.0837E-04
605	2	605.62	5.1408E-03	604.76	3.9428E-03		
615	1	615.28	1.9162E-03				
620	2	620.87	4.8491E-03	620.35	5.2962E-03		
625	1					626.21	8.9570E-04
630	2	630.07	5.0720E-03	631.88	3.5879E-03		
650	2	648.68	1.8654E-03	649.33	1.8067E-03		
660	1	662.10	3.0397E-03				
665	1			663.64	2.1783E-03		
680	2	681.56	2.5200E-03	681.37	2.6828E-03		
690	1					688.40	8.2200E-04
700	4	700.00	1.3515E-03	699.26	6.3451E-03	700.00	2.1985E-03
		698.91	4.3662E-03				
Total # Peaks		20		20		10	

Table 3. List of characteristic spectral peak centers and amplitudes for (second-order derivatives of) 3 seagrasses found within the coastal waters of metro-Adelaide, South Australia.

5 nm-increments	# overlapping	2nd deriv. for *Posidonia spp.*		2nd deriv. for *Amphibolis spp.*		2nd deriv. *for Heterozostera spp.*	
		Center	Amplitude	Center	Amplitude	Center	Amplitude
400	2			400.49	3.4000E-05	400.00	5.0395E-04
410	1			412.27	2.2000E-05		
415	1	414.08	1.4104E-04				
435	3	433.72	2.9124E-04	433.97	1.3747E-04	435.90	2.1496E-04
450	2	451.52	2.8376E-04	450.11	1.1605E-04		
470	3	469.51	5.6083E-04	469.35	2.6845E-04	469.34	3.5719E-04
485	1	486.51	3.7610E-04				
490	1			488.26	1.7307E-04		
495	1					497.44	1.7493E-04
505	3	503.00	6.0498E-04	503.32	4.0744E-04	505.73	8.3300E-05
515	3	516.03	6.2241E-04	516.95	2.8658E-04	515.02	2.6084E-04
520	2	520.17	2.1410E-04	520.19	7.5500E-05		
535	1	534.63	2.2880E-04				
540	2	541.69	4.8892E-04	538.59	7.9300E-05		
545	2			542.56	2.4259E-04	543.06	9.4000E-05
550	3	552.37	4.1716E-04	552.36	1.9494E-04	550.86	5.4600E-05
565	1			564.80	1.0734E-04		
570	3	568.84	1.0396E-03	569.66	7.1136E-04	570.63	1.2365E-04
575	1			574.19	1.0723E-04		
585	3	586.13	9.2022E-04	583.46	1.2898E-04		
				587.12	3.1183E-04		
590	1			590.40	8.5400E-05		
595	1					596.03	5.0600E-05
600	2	601.70	9.4765E-04	600.86	4.3392E-04		
605	1			604.67	1.3125E-04		
615	2	616.60	9.6372E-04	615.80	6.6285E-04		
620	1					617.59	6.5800E-05
625	1			626.65	2.7605E-04		
630	2	628.00	4.1468E-04	630.26	8.6000E-05		
640	1			640.91	9.9100E-05		
645	2	644.08	8.1952E-04	644.96	4.8734E-04		
650	1			651.81	2.6689E-04		
660	2	658.27	7.7875E-04	660.04	5.1615E-04		
670	1			669.10	1.1487E-04		
675	2	677.48	7.6578E-04	676.09	5.6087E-04		
680	1			681.62	1.0372E-04		
690	2	692.44	2.8689E-04			688.61	9.9000E-05
695	3	695.98	1.0342E-03	694.46	1.7778E-04		
				693.73	8.5915E-04		
700	1			700.00	1.2146E-04		
705	1	703.67	6.9153E-04				
Total # Peaks		22		33		12	

Table S4. List of characteristic spectral peak centers and amplitudes for (fourth-order derivatives of) 3 seagrasses found within the coastal waters of metro-Adelaide, South Australia.

5 nm-increments	# overlapping	4th deriv. for *Posidonia spp.*		4th deriv. for *Amphibolis spp.*		4th deriv. for *Heterozostera spp.*	
		Center	Amplitude	Center	Amplitude	Center	Amplitude
395	1					394.46	4.5300E-06
405	1	404.77	3.3000E-06				
420	2	421.97	5.9300E-06			417.66	4.3500E-06
425	1			423.80	5.4100E-06		
445	2	443.37	1.0200E-05			444.15	2.7400E-06
460	3	459.73	1.4500E-05	459.47	1.0300E-05	458.39	3.7700E-06
480	1	478.33	1.6700E-05				
490	2			487.77	1.5800E-05	489.37	4.5400E-06
495	1	494.26	1.8600E-05				
510	3	512.41	5.2700E-06			511.11	3.4300E-06
		508.32	1.8400E-05				
515	1			513.31	1.1700E-05		
520	1					518.80	-1.4700E-06
525	1	526.99	3.7000E-05				
530	2			529.92	1.7800E-05	530.74	8.8000E-06
545	1	544.93	2.2700E-05				
555	2			553.51	2.2700E-05	556.68	4.4800E-06
560	2	558.87	3.5300E-05	559.66	9.0800E-06		
565	1	562.87	1.1800E-05				
570	1					570.86	-7.9700E-07
575	1	574.34	1.1600E-05				
580	2	578.46	4.4600E-05	578.94	1.5700E-05		
585	2			586.05	1.0600E-05	582.54	5.1200E-06
595	1	593.90	4.6300E-05				
605	2			603.59	1.4600E-05	603.43	6.4600E-06
610	1	609.16	4.0900E-05				
625	1	624.21	3.0200E-05				
630	2			629.08	3.0000E-05	630.02	3.9700E-06
635	1	635.81	4.9500E-05				
650	2	652.09	3.5800E-05			648.29	-1.7800E-06
660	1					661.57	3.7600E-06
665	1			664.57	2.8500E-05		
670	1	668.13	4.4800E-05				
680	1					681.74	3.0300E-06
685	2	686.11	5.3800E-05	685.67	2.4100E-05		
695	1					692.72	-1.5700E-06
700	1	700.11	2.0700E-05				
710	1			711.08	1.9200E-05		
Total # Peaks		22		14		17	

Table S5. Five steps for differentiation of benthic bottom types found in South Australia study area.

<u>Step 1</u>: **Separate sand from all vegetative benthic bottom types either grossly or finely.** • Based grossly on simple visual assessment, it is apparent that the maximum reflectance value of sand throughout the visible light spectrum is higher by an entire order of magnitude from that of any seagrass species. • Alternatively, sand can be finely separated from a vegetative benthic bottom type by calculating the 566:689 band ratio, wherein a value of this ratio of less than or more than +1.00 indicates the absence or presence of the red-edge characteristics of any type of benthic vegetation, respectively.
<u>Step 2</u>: **Separate algae and detritus from other submerged aquatic vegetation.** • Refers to utilization of the 566:600 band ratio. • Due to the dampening of green reflectance and amplification of red reflectance, a ratio of $\leq +1.00$ may help indicate the presence of green algae and/or seagrass leaves biofouled with epiphytes. However, it is important to note that this green to red ratio characteristic, as additionally reported by (O'Neill et al., 2011), could not separate green algae from seagrasses that are potentially ridden with epiphytes.
<u>Step 3</u>: **Verify that this group of all other submerged aquatic benthic vegetation specifically refers to the three dominant seagrasses observed within this study.** • There were three specific instances (507, 575, and 700 nm) in which all three seagrasses each contained a characteristic first-order derivative peak at relatively the same 5-nm wavelength. These characteristic peaks, and differences in their relative magnitude trends, at these particular wavelengths are in fact characteristic of dominant seagrasses within the study area. • Specifically, although occurring throughout the visible light spectrum, it is most apparent when observing the relative magnitudes at these particular 5-nm wavelengths that there is a trend of highest to lowest reflectance of seagrasses, that is: *Posidonia* > *Amphibolis* > *Heterzostera spp*.
<u>Step 4</u>: **Separate *Heterozostera* from *Posidonia* and *Amphibolis* seagrasses.** • This can be done by utilizing the multiple wavebands throughout the visible light spectrum in which *Posidonia* and *Amphibolis* are characterized as overlapping. Specifically, this can be done by potentially utilizing three levels of increasing criteria thresholds for separation of *Heterozostera*. • <u>Lowest cost/effort</u>: At the lowest and most gross criteria threshold, there is one instance at 492 nm in which only *Heterozostera* seagrass had a zero-order distinguishing spectral peak. Additionally, *Heterozostera* had relatively the lowest reflectance magnitude, regardless of zero, first, second, or fourth-order derivative analyses. • <u>Moderate cost/effect</u>: At the medium and likely more useful criteria threshold, first-order derivative peaks can be used; specifically, there were eleven instances (417, 456, 474, 491, 522, 590, 605, 621, 631, 649, and 681 nm) throughout the visible light spectrum where only *Posidonia* and *Amphibolis* contained characteristic first-order derivative peaks within the same wavelength. This information can be used in conjunction with the fact that *Heterozostera* continually had the lowest reflectance throughout the visible light spectrum, and this is most evident when assessing relative neighboring amplitudes at the first-order derivative, where *Heterozostera* had a consistent relative reflectance with a median of 0.18. Although, if there are time-constraints for utilization and application of all eleven wavelengths, at least three of these regions should be used. For separation of *Heterozostera* from both *Posidonia* and *Amphibolis*, it is suggested (due to possible time and funding constraints that are common) that at least three major regions at 455, 525, and 605 nm be targeted since they represent the boundaries of the UV-visible light, acute green, and red edge regions, respectively. • <u>Highest cost/effort</u>: The highest and finest level of criteria threshold for isolation of *Heterozostera* is most useful for studies that can utilize aerial or satellite imagery throughout the visible light spectrum. So with less constraints for time and funding, one can therefore isolate first-order derivative peaks at ten specific wavelengths (444, 478, 506, 525, 549, 577, 599, 626, 688, and 700 nm) which were found to characterize *Heterozostera* within this study.

<u>Step 5</u>: Separate *Posidonia* from *Amphibolis*.

- Using the first-derivative analyses, there are two short bandwidths at which *Posidonia* can be distinguished from *Amphibolis*.
- The first bandwidth occurs at the border for UV-visible light at around 435-440 nm, and the second borders along the falling limb of the acute green peak at around 540-555 nm. Specifically, within the UV-visible light region, the first-derivative peak that characterizes *Posidonia* at 439 nm compared to that of *Amphibolis* at 437 nm occurs at a stronger reflectance value that is an order of magnitude higher (3.46×10^{-03} and 9.29×10^{-04}, respectively).
- Then, within the descending limb of the acute green peak, *Posidonia* can again be distinguished from *Amphibolis* due to it continually containing stronger reflectance values of first-derivative peak amplitudes. Specifically, first-order derivative spectral peaks that characterized *Posidonia* measured at 542 and 556 nm, and had reflectance values of 7.82×10^{-03} and 5.43×10^{-03}, respectively; whereas *Amphibolis* at 545 nm is characterized by having a reflectance of 5.04×10^{-03}, and allowing for possible differentiation between the two most commonly found dominant seagrasses within the study area.

Table S6. Wavelengths at which reflectance of seagrass measured in filtered marine water (Whatman GF/C 1.2 μm glass microfiber filter, 90 mm diameter) were significantly higher than those in unfiltered marine water.

wavelength (nm)	significant p-value (p<0.001)	mean seagrass reflectance, filtered water	mean seagrass reflectance, unfiltered water	wavelength (nm)	significant p-value (p<0.001)	mean seagrass reflectance, filtered water	mean seagrass reflectance, unfiltered water
350	9.132E-32	0.00382566	0.00182701	387	1.45E-168	0.0073162	0.0026577
351	5.694E-41	0.0039607	0.00189668	388	1.97E-139	0.0074867	0.0025862
352	8.463E-41	0.00411289	0.00201561	389	6.99E-168	0.007456	0.0025867
353	2.997E-45	0.00405797	0.00191033	390	9.96E-242	0.0085491	0.0028533
354	1.366E-38	0.00452499	0.00203946	391	2.92E-321	0.0095783	0.003093
355	0.0003021	0.00484155	0.00195385	392	7.21E-282	0.0100386	0.0031291
356	2.132E-55	0.00469753	0.00214583	393	1.59E-225	0.0082645	0.0028313
357	1.245E-41	0.00441869	0.00199894	394	2.74E-133	0.0062442	0.0024667
358	2.051E-36	0.00390866	0.0021549	395	9.35E-222	0.0081387	0.0028656
359	2.915E-05	0.00375242	0.00630734	396	8.69E-257	0.0097386	0.0032679
360	3.335E-08	0.00460378	0.00867366	397	5.723E-86	0.008075	0.0037622
361	0.0001138	0.00502196	0.00218733	398	5.883E-40	0.0084705	0.0047426
362	2.053E-55	0.00463582	0.00216082	723	3.53E-276	0.1173458	0.0987649
363	2.907E-59	0.00484059	0.00213451	724	1.77E-281	0.1126915	0.0945879
364	1.896E-80	0.00522747	0.00203803	725	1.47E-294	0.1076516	0.0897723
365	4.035E-09	0.00544676	0.00914861	728	3.05E-302	0.102778	0.0852829
366	4.687E-06	0.00596044	0.00440697	729	7.64E-321	0.1025059	0.0848919
367	7.14E-106	0.00628894	0.00223947	730	1.17E-284	0.0998564	0.0833072
368	2.17E-110	0.00634275	0.00235344	731	6.73E-279	0.0988036	0.0826041
369	8.68E-115	0.00624825	0.00235787	732	1.03E-278	0.0986252	0.0824345
370	5.26E-134	0.00657948	0.00235841	733	7.32E-252	0.0998646	0.0843076
371	1.24E-136	0.00654214	0.00224874	734	1.84E-246	0.1011015	0.0856077
372	8.11E-101	0.00665623	0.00239252	735	1.67E-230	0.1009012	0.08599
373	2.31E-120	0.00625107	0.00224569	736	7.81E-229	0.0987207	0.0841625
374	2.18E-103	0.00573942	0.00220277	737	3.01E-310	0.0965919	0.0821977
375	6.6E-100	0.00566004	0.00228781	738	4.04E-228	0.0953598	0.0813041
376	0.0012901	0.00627214	0.00816792	739	5.64E-235	0.0938057	0.079797
377	2.757E-18	0.0067609	0.004203	740	4.4E-229	0.0926976	0.0790214
378	1.57E-184	0.00773332	0.00253669	741	3.15E-225	0.0921574	0.078699
379	2.89E-204	0.00805049	0.00265429	742	1.67E-215	0.0908761	0.07788
380	5.1E-112	0.00747942	0.00260507	743	5.82E-212	0.090478	0.0776405
381	2.02E-169	0.00759487	0.00263285	744	7.81E-228	0.0907246	0.0776401
382	4E-166	0.00723735	0.0025055	745	3.63E-211	0.0892859	0.0766648
383	1.93E-110	0.00586182	0.00227578	746	1.36E-261	0.0883103	0.0760821
384	1.136E-60	0.00539586	0.00218404	747	4.94E-218	0.0876626	0.0750796
385	1.38E-125	0.00632245	0.00235905	748	8.59E-213	0.0868448	0.074551
386	1.52E-171	0.00708166	0.00254603	749	2.27E-216	0.0857941	0.0735802